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Oxygen-evolving diatom thylakoid membranes

Tracey A. Martinson [a], Masahiko Ikeuchi [b], F. Gerald Plumley [a,*]

[a] *Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, AK 99775-7220, USA*

[b] *Department of Biology, University of Tokyo, Komaba 3-8-1, Meguro, Tokyo 153, Japan*

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Abstract

Two protocols were developed that yielded purified oxygen-evolving thylakoid membranes from the diatom *Cylindrotheca fusiformis*. One protocol employed sonication, while the second involved French press lysis of protoplasts formed by brief culture of cells in a cation-depleted medium. Regardless of the method of cell breakage, some damage to electron transport components occurred. For preservation of both light-dependent electron transport activity and in vivo fluorescence properties, 2 M sorbitol proved to be more effective than 1 M sorbitol, regardless of the method used for cell lysis. Thylakoids purified in 2 M sorbitol using the protoplast/French press method showed the best preservation of in vivo fluorescence emission signals and Photosystem II activity with ferricyanide was completely inhibited by DCMU. Thylakoids purified in 2 M sorbitol using sonication had higher rates of Photosystem II activity with ferricyanide, but this activity was less sensitive to DCMU. Whole-chain electron transport activity was low in all preparations. This is the first report of O_2 evolution and of long-wavelength fluorescence in purified thylakoids of any chromophytic alga.

Keywords: Diatom; Thylakoid; Oxygen evolution; Photosystem I; Photosystem II; Fluorescence

1. Introduction

Diatoms are ecologically important in both freshwater and marine ecosystems, but in spite of their significance, little is known about the biochemistry of photosynthesis in these algae. Although immunological [1,2] and deduced protein sequence data [3,4] indicate that the major photosynthetic proteins of diatoms are homologous to those of green plants/algae and cyanobacteria, there are reasons to suspect that diatoms will exhibit some unique structure–function relationships. The thylakoid membranes of diatoms are arranged in stacks of three and are not segregated into granal and stromal lamellae as in green plants/algae [5]. Immunocytochemical studies with the diatom *Phaeodactylum tricornutum* [6] showed that Photosystem I (PSI) is present in both appressed and unappressed regions of the thylakoids, which is in contrast to the lateral heterogeneity observed in thylakoids of green plants [7]. Moreover, the ease with which in vivo fluorescence signatures are lost when diatom cells are lysed [8] suggests that the molecular interactions between pigment–protein complexes in diatoms may differ from those in green plants and cyanobacteria. Unlike green plants, diatoms contain Cyt c_{550} [3,9] and immunological studies indicate that homologs of the 23- and 17-kDa polypeptides of the green plant water oxidation com-

* Corresponding author. Fax: +1 (907) 474-7204; E-mail: fffgp@uaf.edu

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plex are absent (Martinson and Plumley, unpublished data). These observations suggest that the water oxidation complex of diatoms is more similar to that of cyanobacteria [10,11] and red algae [12] than it is to the green plant complex [13]. Lastly, Cyt c_{553} functions as electron carrier between the Cyt b_6/f complex and PSI in these algae [14], and it is likely that the acceptor side of diatom PSI may differ structurally from that of green plants, which have plastocyanin [15]. Detailed studies of diatom Photosystem II (PSII) and PSI have been blocked at the level of obtaining purified thylakoids that are capable of O_2 evolution and/or partial electron transport. A major stumbling block in working with these algae has been the difficult nature of breaking open the silica frustule surrounding the diatom cell without damaging intracellular structures.

In this communication, we report two protocols for purifying O_2-evolving thylakoids from a diatom. One employs sonication as a means of breaking cells, while the other utilizes the ability of some diatoms to form protoplasts under certain growth conditions which, in turn, are readily lysed at low pressure in a French press. The biochemical properties and spectroscopic features of thylakoid preparations obtained using these two methods with buffers of differing osmotic potential were compared in an attempt to define the optimum conditions for purifying O_2-evolving thylakoids from diatoms. Here, we present an initial characterization of the electron transport activities, biochemical properties, and spectroscopic features of these thylakoids. The results indicated that some damage to electron transport components occurred with either method of cell breakage. These protocols are a useful starting point for the purification of O_2-evolving thylakoids from other diatoms and chromophytes.

2. Materials and methods

2.1. Cultures and growth conditions

Cultures (3 l) of *Cylindrotheca fusiformis* (Watson's strain [13]), were grown in 4-l Pyrex flasks in artificial seawater (ASW) medium as described [2]. Cultures were illuminated using two cool-white fluorescent bulbs (110 μEin m^{-2} s^{-1}) with continuous stirring and aeration using an air supply supplemented with CO_2.

2.2. Formation of protoplasts and purification of thylakoid membranes using French press disruption

Cells ($\approx 5\times10^5$ cells ml^{-1}) were harvested by centrifugation at $2000\times g$ for 10 min, aseptically transferred to 300 ml protoplast medium (PPM; slightly modified from [16]; 0.5% yeast extract, 1% peptone, and 3% NaCl, plus vitamins, phosphate and nitrate as per ASW), and incubated in the light with shaking for 24 h. After centrifugation at $2000\times g$ for 10 min, cells were resuspended in 40 ml ice-cold Buffer A (20 mM potassium phosphate pH 8, 5 mM ε-aminocaproic acid (ACA), 1 mM benzamidine (BAM)). Protoplast formation, verified microscopically, was typically 45–50%. During thylakoid preparation, no attempt was made to separate protoplasts from unlysed cells.

For purification of thylakoids, all buffers contained 20 mM potassium phosphate pH 8, 5 mM ACA, 1 mM BAM, plus other components as noted below, and all steps were carried out on ice or at 4°C. Protoplasts and unlysed cells were pelleted at $750\times g$ for 10 min, resuspended in 10 ml Buffer B (2 M sorbitol, 1 mM $MgCl_2$), and incubated for 1 h in darkness at 0°C. After passage through a chilled French press at 2000 psi, unbroken protoplasts and cells were pelleted and resuspended as before, and passed through the French press again. The combined supernatants were centrifuged at $39\,500\times g$ for 15 min, resuspended in 10 ml Buffer C (2 M sorbitol), and homogenized using a tight-fitting motor-driven Teflon pestle (providing a crude membrane fraction). After diluting to 30 ml with Buffer C, and pelleting by centrifugation at $39\,500\times g$ for 15 min, the membrane fragments were resuspended in 20 ml Buffer D (3.8 M sorbitol) and homogenized. The solution was transferred to four 13-ml ultracentrifuge tubes, overlaid with 4 ml Buffer E (3.2 M sorbitol) and 4 ml Buffer F (2.5 M sorbitol). Thylakoids were purified by flotation in a Beckman SW-40 swinging bucket rotor ($284\,000\times g$, 45 min), recovered from the E/F interface, diluted with two volumes of Buffer A, and pelleted ($39\,500\times g$, 15 min). Pellets were resuspended in a small volume of Buffer

C, and chlorophyll (Chl) concentrations were determined in 90% acetone using the equations of Jeffrey and Humphrey [17]. Yields were typically 40–50% (calculated on a Chl basis). Freshly prepared thylakoids were used for all experiments.

2.3. Purification of thylakoids using sonication

Cells (10^6 cells ml^{-1}) were harvested by centrifugation at $2000 \times g$ for 10 min and resuspended in 20 ml Buffer 1 (1 or 2 M sorbitol, 20 mM MES-NaOH (pH 6), 5 mM ACA, 1 mM BAM, 1 mM $MgCl_2$). Cells were broken with a sonic probe (1.25 cm diameter, Bronwill Scientific) in 10-ml aliquots using two 15-s bursts at 105 W. Cells were kept on ice during sonication and cooled for 30 s between bursts. Unbroken cells and debris were pelleted ($750 \times g$, 5 min). The supernatants were set aside and the pellets resuspended in 20 ml Buffer 1 and subjected to sonication; >95% breakage was obtained with six to eight cycles of sonication/centrifugation. The supernatants were combined, centrifuged ($39\,500 \times g$, 15 min), and the crude membrane pellets resuspended in 10 ml Buffer 2 (1 or 2 M sorbitol, 20 mM MES-NaOH (pH 6), 5 mM ACA, 1 mM BAM). After homogenization with a motor-driven Teflon pestle and dilution to 30 ml with Buffer 2, samples were centrifuged ($39\,500 \times g$, 15 min). Pellets were resuspended in a total of 20 ml Buffer 3 (3.8 M sorbitol, 5 mM MES-NaOH (pH 6), 5 mM ACA, 1 mM BAM), homogenized using a motor-driven Teflon pestle, and transferred to four 13-ml ultracentrifuge tubes. The samples were successively overlaid with 4 ml of Buffer 4 (same as Buffer 3, but with 3.2 M sorbitol), and 4 ml of Buffer 5 (same as Buffer 3 but with 2.5 M sorbitol). Thylakoids were purified by flotation in a Beckman SW-40 swinging bucket rotor ($284\,000 \times g$, 1 h), recovered from the 4/5 interface, diluted with two volumes of Buffer 6 (same as Buffer 3, but without sorbitol), and pelleted ($39\,500 \times g$, 15 min). Pellets were resuspended in a small volume of Buffer 2. Chl concentrations were determined in 90% acetone [17]. Yields were typically 20% (calculated on a Chl basis). Freshly prepared thylakoids were used for all experiments.

It is important to note that excessive sonication (e.g. higher wattages, longer bursts, or more bursts prior to each low-speed centrifugation) frequently resulted in decreased rates of electron transport, severe decreases in yields, and, in some instances, a complete loss of activity (not shown). Sonication conditions need to be optimized for each diatom species.

2.4. Electron transport measurements

O_2 evolution/uptake was measured with a Clark-type electrode (Gilman) at 20°C and a total Chl concentration of 3 μg ml^{-1} (cells) or 6–10 μg ml^{-1} (thylakoids). O_2 evolution by cells was measured in ASW supplemented with 10 mM HCO_3^-. Thylakoids were assayed in either Buffer C or Buffer 2, with additions as noted. Electron transport from water to PSI was measured as O_2 uptake using 100 μM methylviologen (MV). Electron transport activity was also measured as O_2 evolution using either ferricyanide (FeCN; plus 1 mM $MgCl_2$) or freshly sublimed *p*-benzoquinone (BQ). DCMU and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) were used at 20 μM and 1 μM, respectively. PSI-dependent O_2 uptake (Mehler reaction) assays included 300 μM ascorbate, 10 μM 2,6-dichlorophenol-indophenol (DCPIP), 20 μM DCMU, 10 mM methylamine, 5 mM KCN, and 100 μM MV. Saturating red light (180 μEin m^{-2} s^{-1}, 660 nm with 25 nm spectral line half-width; Hansatech) was used for all measurements as suggested by Allen and Holmes [18].

2.5. Spectroscopic analyses

Absorption spectra were obtained with a Hewlett-Packard HP8452A diode array spectrophotometer at 5 μg Chl ml^{-1}. For fluorescence measurements at 77 K, samples containing 1.5 μg Chl ml^{-1} were brought to 50% glycerol and frozen in liquid N_2. Since freezing in liquid N_2 can cause 'glassing' and other problems, we estimated fluorescence yields from multiple analyses of each sample. Spectra were obtained using an Aminco SLM500-C spectrofluorometer with a 4 nm bandpass for both emission and excitation. Emission spectra were corrected for the decrease in detector efficiency using the correction factor supplied with the instrument.

2.6. SDS-PAGE and immunodetection of polypeptides

For denaturing SDS-PAGE, all samples were solubilized in 60 mM Tris-HCl (pH 8.5), 2% lithium dodecylsulfate (LDS), 60 mM DTT, 5 mM ACA, 1 mM BAM, and 12% sucrose. Cells were solubilized at 0.25 mg Chl ml^{-1}, thylakoids at 0.5 mg Chl ml^{-1}. Samples were heated at 100°C for 1–2 min (unless otherwise noted; e.g. for cytochrome staining), centrifuged (16 000 × *g*, 5 min), and 10 μg Chl loaded per lane. Electrophoresis was performed on 10–20% polyacrylamide gels with a 4-cm stacking gel of 5% polyacrylamide [2,19]. The reservoir buffer contained 50 mM Tris Base and 383 mM glycine, with 0.1% SDS in the upper buffer (modified from [20]). Electrophoresis was carried out at 4°C for 16–18 h at 16 mA. Cytochromes were stained with 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMBZ; [21]) or Coomassie brilliant blue G-250 [22].

Immediately following denaturing SDS-PAGE, proteins were transferred to nitrocellulose using a semi-dry transfer apparatus (Bio-Rad) at 4°C for 1.5 h at 20 V. After transfer, the nitrocellulose was stained with Ponceau S [23] and air dried. Blots were reacted with antibodies to either the PSI complex from cyanobacteria, the CP47, CP43, D1, D2, or 33-kDa polypeptides from spinach, the large [24] or small subunit (Plumley, unpublished) of Rubisco from *Cylindrotheca fusiformis*, the β-subunit of ATP-ase from romaine lettuce [25], or a *Chlamydomonas* 29-kDa light-harvesting polypeptide (LHCP) [2,25], and developed using alkaline phosphatase-conjugated goat anti-rabbit IgG in conjunction with 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium [23].

3. Results

3.1. Methods of cell disruption

As cell breakage of diatoms represents a significant obstacle for purification of subcellular components, we initiated this work by investigating two methods of cell breakage, French press disruption and sonication. Cell breakage in a French press required two passes at ≈18 000 psi [2]. Cells could be broken at 10 000 psi if they were first subjected to a freeze–thaw treatment. However, thylakoids purified from cells broken at high pressure and/or that had been frozen completely lacked electron transport activity and showed marked changes in the 77K fluorescence emission spectrum (not shown). Cells were readily broken when subjected to sonication, and thylakoids purified using this method retained the capacity for O_2 evolution [26]. However, since sonication is known to cause loss of plastocyanin from spinach [27] and cyanobacterial [28] membranes, we sought to develop a more gentle means of cell breakage. In doing this, we capitalized on the observation that a number of diatom species can be induced to form protoplasts when cultured in media lacking Mg^{2+}, Ca^{2+}, silicic acid, and/or Mn^{2+} ([16] and references therein). For *Cylindrotheca*, we developed a method based on that used for *Nitzschia alba* [16,29]. In our method, cells grown in ASW were aseptically transferred to protoplast medium and incubated at room temperature with shaking for 24 h. Rates of O_2 evolution and 77K fluorescence properties were unaffected by this treatment (not shown). Subsequent transfer of cells to either ddH_2O or a buffer lacking an osmoticum led to formation of protoplasts, which were readily lysed at low pressure (2000 psi) in a French press. Protoplasts did not form in solutions that lacked an osmoticum but contained high concentrations of salts (e.g. 0.5 M K-phosphate, 0.3 M K-citrate; [8]). Both the sonication and protoplast/French press methods were developed further.

3.2. Optimization of thylakoid isolation protocols

Preliminary data indicated that preservation of O_2 evolution in purified thylakoids required attention to two factors: the conditions of cell breakage and the osmotic concentration of the buffers used in the protocol. To determine the optimal buffer composition for purifying thylakoid membranes, we first assayed the PSII activity of crude membranes prepared using the sonication method (Table 1). No O_2 evolution was observed without an added electron acceptor, regardless of buffer conditions. However, we found high concentrations of sorbitol to be effective in preserving photosynthetic electron transport activity from H_2O to BQ; high (>1 M) concentrations of sucrose were also effective (not shown) but it was more troublesome to handle. Rates of O_2 evolution

Table 1
Optimization of thylakoid purification protocols

Method	Type of preparation	Buffer type and pH[a]	Osmoticum	0.5 mM BQ
Sonication	Crude membranes	MES-NaOH, pH 6	0.5 M sorbitol	9.6[b]
		MES-NaOH, pH 6	1.0 M sorbitol	12.1
		MES-NaOH, pH 6	1.5 M sorbitol	16.3
		MES-NaOH, pH 6	2.0 M sorbitol	18.1
		MES-NaOH, pH 6	2.5 M sorbitol	11.5
		MES-NaOH, pH 6	3.0 M sorbitol	12.8
		Na-phosphate, pH 7	2.0 M sorbitol	17.7
		Tris-HCl, pH 8.5	2.0 M sorbitol	9.6
Protoplast/French press	Protoplasts	None (ddH_2O)	None	12.5 ± 1.1
		MES-NaOH, pH 6	None	8.9 ± 2.3
		HEPES-NaOH, pH 7.5	None	34.9 ± 4.7
		K-phosphate, pH 8	None	53.2 ± 3.0
	Crude membranes	K-phosphate, pH 8	1.0 M sorbitol, 1.0 M glycine betaine	26.3 ± 3.3
		K-phosphate, pH 8	2.0 M sorbitol	30.8 ± 4.6
		K-phosphate, pH 8	2.0 M sorbitol, 1.0 M glycine betaine	25.2 ± 6.6
		0.5 M K-phosphate, 0.3 M K-citrate, pH 7.2[c]	2.0 M sorbitol	28.0 ± 3.5

[a]Buffers were used at 20 mM unless otherwise noted.
[b]Rates are given as μmol O_2 mg Chl^{-1} h^{-1}.
[c]Protoplasts were formed in 20 mM K-phosphate pH 8.

reached a peak at 2 M sorbitol (Table 1). Other studies with crude membranes tested the effects of different buffers/pH and showed that both MES-NaOH pH 6 and Na-phosphate pH 7 were suitable for use in the sonication procedure, while Tris gave reduced rates of O_2 evolution by crude membranes (Table 1). Based on these studies, we chose to use 2 M sorbitol with 20 mM MES-NaOH pH 6 as the buffer for the sonication method. We chose to use BQ in subsequent assays as other quinones (e.g. 2,6-dichloro-*p*-benzoquinone) did not give reproducible results with these diatom preparations (not shown). Since BQ is a membrane-permeable acceptor, it offered the additional benefit of serving as an internal standard in that it permitted a direct comparison between thylakoids, cells, and protoplasts.

In developing the protoplast/French press method, we tested different buffer components at two points during the thylakoid isolation procedure: protoplasts and crude membranes. Protoplasts and unlysed cells were separated for these assays by low speed centrifugation ($250 \times g$, 5 min) so that O_2 evolution from intact cells would not confound the results. Protoplasts did not evolve O_2 unless supplemented with an electron acceptor such as BQ (Table 1). The highest rates were obtained using 20 mM K-phosphate pH 8 (Table 1). Higher concentrations of buffer (i.e. >20 mM K-phosphate) led to significantly decreased formation of protoplasts and were not tested for O_2 evolution. For the next step, protoplasts formed in 20 mM K-phosphate pH 8 were lysed in buffers of various osmotic strengths. In these crude membrane preparations, rates of O_2 evolution with BQ were $\approx 60\%$ of the rate obtained for protoplasts, and were largely independent of the sorbitol concentration (Table 1). Neither the addition of glycine betaine nor the use of high ionic strength buffers enhanced the preservation of activity (Table 1). From these data, we opted to use 2 M sorbitol with 20 mM K-phosphate pH 8 when purifying thylakoids using the protoplast/French press method.

3.3. Electron transport characteristics of different thylakoid preparations

To optimize the protocol for purifying O_2-evolving

thylakoids from diatoms, we compared the properties of three different thylakoid preparations (Table 2). Two preparations were isolated using the sonication method, one with 1 M sorbitol/MES-NaOH pH 6 and the other with 2 M sorbitol/MES-NaOH pH 6. A third preparation was made using the protoplast/French press method with 2 M sorbitol/K-phosphate pH 8. Experiments with each type of preparation were repeated at least three times.

Electron transport properties of cells and purified thylakoids were measured as changes in O_2 levels in the presence of various electron donors/acceptors/inhibitors and are summarized in Table 2. A model showing the locations of electron donation to different acceptors and the sites of action for DCMU and DBMIB is shown in Fig. 1. No changes in O_2 concentration were detected upon illumination of any of the thylakoid preparations in the absence of added electron acceptors. With 0.5 mM BQ, rates of O_2 evolution were highest for thylakoids purified using 2 M sorbitol ($\approx$20–25 μmol O_2 mg Chl^{-1} h^{-1}). These rates were only about 21–26% of the rates observed for cells with 0.5 mM BQ (Table 2). Thylakoids purified using 1 M sorbitol had very low rates of O_2 evolution with 0.5 mM BQ (Table 2). DCMU completely inhibited O_2 evolution in all preparations when BQ was used as acceptor. Rates of O_2 evolution reached a maximum at 3 mM BQ for thylakoids, and 0.5 mM BQ for cells. Overall, the maximum rate of O_2 evolution for thylakoids with BQ was only about 50% of the maximum rate obtained for cells with BQ. We concluded from these results that, with BQ as electron acceptor, the activities of thylakoids purified in 2 M sorbitol were about the same, regardless of the method used for cell breakage, while use of 1 M sorbitol was detrimental to preserving the capacity for O_2 evolution.

With 2 mM FeCN as electron acceptor, the highest rates of O_2 evolution were obtained for thylakoids purified in 1 M sorbitol using sonication (Table 2). Rates were somewhat lower for thylakoids purified in 2 M sorbitol using sonication, and the lowest rates

Table 2
Electron transport rates in various thylakoid preparations

Preparation	Method	No additions	0.5 mM BQ	3 mM BQ	2 mM FeCN	100 μM MV	Ascorbate, DCPIP, MV
		(+DCMU) (+DBMIB)	(+DCMU)	(+DCMU) (+DBMIB)	(+DCMU) (+DBMIB)	(+DCMU) (+DBMIB)	
Cells	In ASW with 10 mM HCO_3^-	72 ± 1.64[a] (0) (0)	94.0 ± 0.21 (0)	93.0 ± 0.1 (0) (ND)	ND[b]	ND	ND
Thylakoids	Sonication in 1 M sorbitol, 20 mM MES-NaOH, pH 6	0 (ND) (0, 0, 0, 0)[c]	5.7 ± 0.5 (0)	21.8 ± 2.8 (0) (ND)	70.0 ± 4.3 (36.5 ± 2.3) (44.5 ± 5.0)	0	−19.4 ± 5.2
	Sonication in 2 M sorbitol, 20 mM MES-NaOH, pH 6	0 (ND) (0, 0, 0, 0)	20.4 ± 0.8 (0)	51.7 ± 0.7 (0) (39.6 ± 0.1)	57.3 ± 2.3 (12.7 ± 2.5) (35.7 ± 5.6)	−10.2 ± 2.2 (0)[d] (0)	−9.5 ± 1.0
	French press of protoplasts at 2000 psi in 2 M sorbitol, 20 mM K-phosphate, pH 8	0 (ND) (0, 0, 0, 0)	23.5 ± 4.2 (0)	52.0 ± 4.6 (0) (35.0 ± 4.2)	40.0 ± 6.8 (0) (25.0 ± 4.6)	0	−16.8 ± 1.7

[a]Rates are given as μmol O_2 mg Chl^{-1} h^{-1}.
[b]Not determined.
[c]Rates with DBMIB in thylakoids were determined at 1, 10, 20, and 50 μM.
[d]Thylakoids also exhibited whole-chain electron transport from water to ferredoxin (−11.8 ± 2.4). O_2 uptake is expected unless $NADP^+$ is added [18]. With 2 mM NADP, O_2 uptake was reduced to −7.4 ± 0.2, suggesting that either a small amount of ferredoxin-$NADP^+$ oxidoreductase is present or that the red algal ferredoxin does not interact effectively with the diatom oxidoreductase.

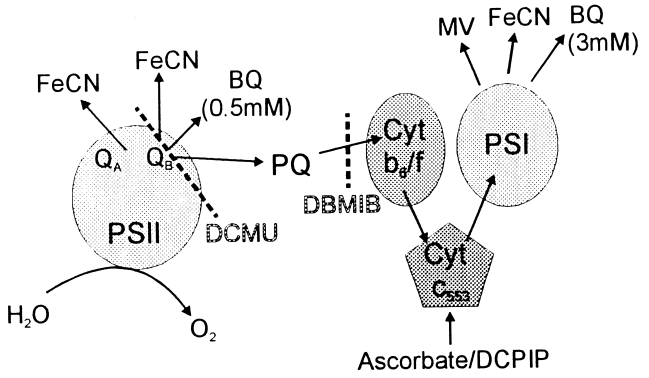

Fig. 1. Diagram of the photosynthetic electron transport chain showing sites of action for various endogenous electron donors and acceptors. (Compiled from [30, 37, 41–43].)

were observed for thylakoids purified in 2 M sorbitol using the protoplast/French press method (Table 2). Only thylakoids purified in 2 M sorbitol using the protoplast/French press method showed complete sensitivity to DCMU (Table 2). DCMU inhibited 47 and 77% of FeCN-dependent O_2 evolution in thylakoids purified using sonication in 1 or 2 M sorbitol, respectively. Since FeCN can serve as both an acceptor for both PSII and PSI [30], the level of inhibition by DBMIB was determined as a means of assessing the partitioning of electron flow to FeCN (Fig. 1, Table 2). DBMIB did not completely inhibit O_2 evolution in any of the preparations, indicating that some electron flow to FeCN through PSII was occurring. DBMIB alone did not support O_2 evolution in any preparation (Table 2) despite its ability to accept electrons in green plant PSII [30,31].

Electron transport was also measured as O_2 uptake using MV, which accepts electrons from PSI [30]. Only thylakoids purified in 2 M sorbitol using sonication exhibited O_2 uptake when supplemented with MV (Table 2). A similar level of activity was observed in these thylakoids with red algal ferredoxin. Both DCMU and DBMIB completely inhibited activity with either acceptor (Table 2). All three thylakoid preparations had low rates of O_2 uptake with MV when ascorbate and DCPIP (10 μM) were included in the assay as donors to Cyt c_{553} (Fig. 1); the lowest rate was observed for thylakoids purified in 2 M sorbitol using sonication (Table 2). Higher concentrations of DCPIP (100 μM) resulted in high rates of non-photochemical O_2 uptake; these high rates led to such sharp reductions in O_2 concentrations that accurate measurements of light-dependent rates could not be obtained (not shown).

3.4. Spectroscopic characterization of different thylakoid preparations

Room temperature absorption spectra (Fig. 2) for each of the purified thylakoid membrane preparations exhibited major peaks that we attribute to Chl *a* (438 and 676 nm), and shoulders likely corresponding to Chl *c* (636 and 470 nm) and fucoxanthin (480 nm). The broad region of absorption between 480 and 560 nm is probably due mainly to fucoxanthin, with some contribution from diadinoxanthin and diatoxanthin [32]. A comparison of the three thylakoid preparations revealed an apparent reduction in carotenoids and Chl *c* in thylakoids prepared using either of the sonication protocols (Fig. 2). This suggested some loss of light-harvesting complexes in the sonication preparations. The organization of Chls and carotenoids in the thylakoid preparations was investigated using and low temperature (77 K) fluorescence spectroscopy (Fig. 3). For comparison, spectra for cells are also shown. When Chl *a* (440 nm) was preferentially excited, cells exhibited fluorescence emission peaks at 685 and 717 nm (Fig. 3). Some variation in the 685/717 ratio for cells is evident, which we attribute to day-to-day variations in cultures and culture conditions. In all thylakoid preparations, fluorescence yields were consistently higher than in cells. The fluorescence yield of thylakoids purified in 1 M sorbitol was ≈120% higher than that of cells (Fig. 3C), while the yields for thylakoids purified in 2 M sorbitol were ≈20% higher

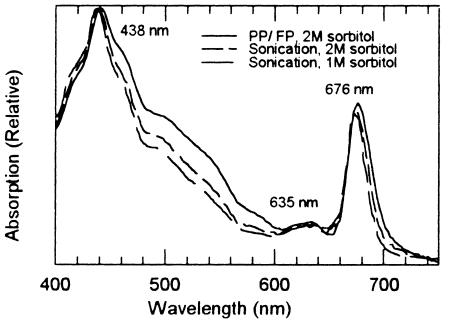

Fig. 2. Absorption spectra for the three thylakoid preparations. Data were normalized to the absorption at 750 nm.

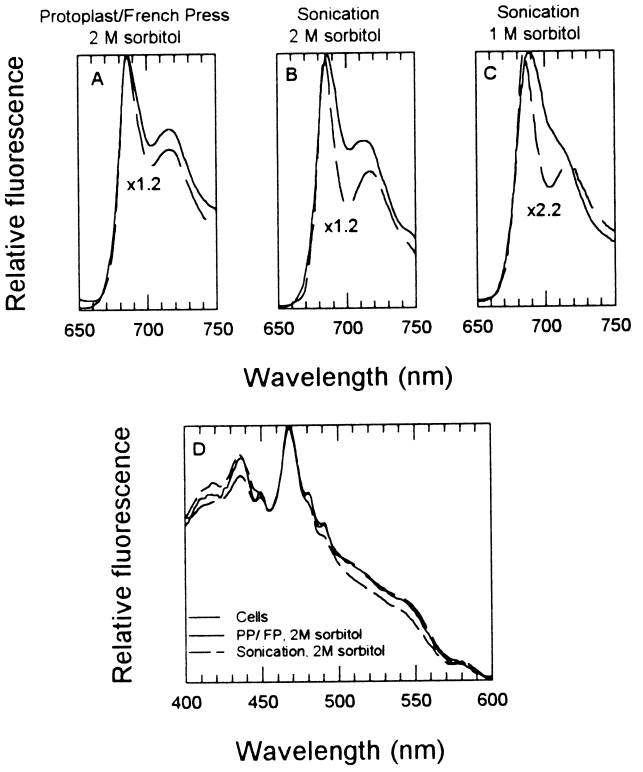

Fig. 3. The 77K fluorescence emission and excitation spectra of cells and purified thylakoids. Emission spectra (440 nm excitation) for cells (dashed line) and thylakoids (solid line) purified using: (A) 2 M sorbitol and protoplast/French press method: (B) 2 M sorbitol and sonication; and (C) 1 M sorbitol and sonication. Excitation spectra for the 685/686 nm emission peaks of cells, thylakoids purified in 2 M sorbitol using sonication, and thylakoids purified in 2 M sorbitol using the protoplast/French press protocol are shown in D.

regardless of the method used for cell breakage (Fig. 3A,B).

To visualize qualitative differences between thylakoids and cells, cell data were normalized to the emission maximum (685–688 nm, depending on the preparation) of thylakoids (Fig. 3). Thylakoids purified in 1 M sorbitol using sonication showed the greatest deviation from in vivo fluorescence properties, with a shift in the major emission peak to 688 nm and a broadening of the fluorescence emission between 690 and 720 nm (Fig. 3C). Sonication in 2 M sorbitol yielded thylakoids with fluorescence emission properties that were more similar to those observed in vivo, with only a slight shift in the peak wavelengths (to 686 and 714 nm), and less broadening at long wavelengths (Fig. 3B). No changes in peak wavelengths were observed for thylakoids purified in 2 M sorbitol using the protoplast/French press method (Fig. 3A), and overall this method gave the best preservation of in vivo fluorescence properties (Fig. 3A). For all thylakoid preparations, the same emission peaks were observed when either Chl *c* (470 nm), or fucoxanthin (490 and 510 nm) were preferentially excited (not shown) indicating that these accessory pigments were energetically coupled to the reaction centers. No short wavelength emissions from uncoupled Chl *a* ($\approx$670 nm) or *c* ($\approx$640 nm) were observed (Fig. 3).

Excitation spectra for the 685/686 (Fig. 3D) and 714/717 nm (not shown) emissions of the thylakoids prepared using 2 M sorbitol were qualitatively identical, as were those of the 685 (Fig. 3D) and 717 nm (not shown) emissions of cells. In both cells and thylakoids, the most substantial contribution to the fluorescence yield appeared to be from Chl *c* (i.e. 470 nm; Fig. 3). These data indicate a high degree of integrity of pigments in the major pigment–protein complexes and suggest the presence of substantial

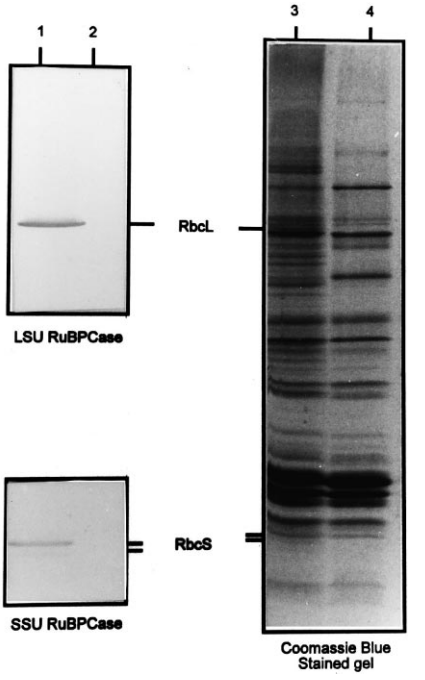

Fig. 4. Cell (lanes 1 and 3) and thylakoid membrane (lanes 2 and 4) proteins resolved by denaturing SDS-PAGE and detected by immunostaining (lanes 1 and 2) or with Coomassie blue (lanes 3 and 4). The immunoblots demonstrate the loss of the large and small subunits of Rubisco in the purified thylakoids. All lanes contained 10 μg Chl. Data shown are for thylakoids purified in 2 M sorbitol using sonication.

light-harvesting components, and, that these properties are retained in thylakoids.

3.5. Identification of photosynthetic polypeptides

The purity of the thylakoid preparations was assessed by denaturing SDS-PAGE and immunoblotting. The three thylakoid preparations were indistinguishable in their polypeptide composition, and only the data for thylakoids purified in 2 M sorbitol using sonication are shown (Figs. 4 and 5). Virtually identical results were obtained when thylakoids were denatured at 70°C for 10 min (not shown), indicating that with sufficiently high SDS:protein ratios, thylakoid proteins from chromophytes do not necessarily aggregate upon heating at 100°C. A comparison of the Coomassie-stained polypeptide profiles of cell extracts (Fig. 4, lane 3) and purified thylakoid membranes (Fig. 4, lane 4) revealed an overall reduction in both the amount and total number of proteins present in thylakoids relative to cells when gels were loaded on an equal Chl basis. Immunoblotting showed the thylakoids to be free of both subunits of Rubisco, an abundant stromal protein that is frequently associated with thylakoids (Fig. 4, lane 2). These results indicate the virtual absence of soluble proteins in the purified thylakoid membranes.

For further confirmation of the purity of the membranes as well as to determine the overall quality of the preparation, we used immunochemical and histochemical procedures to conclusively identify most of the major proteins present (Fig. 5). An antibody to the PSI reaction center polypeptides (PsaA and PsaB) recognized abundant proteins of ≈65 kDa

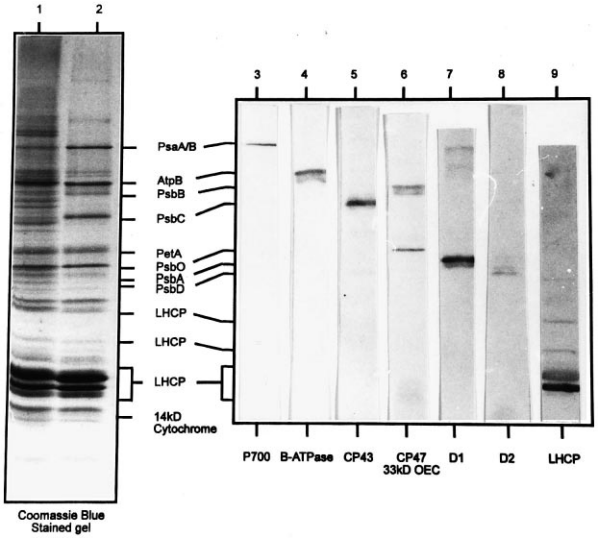

Fig. 5. Immunological identification of photosynthetic proteins in thylakoids purified in 2 M sorbitol using sonication. PsaA/B (lane 3), AtpB (lane 4), PsbC (lane 5), PsbB and PsbO (lane 6), PsbA (lane 7), PsbD (lane 8), and LHCP (lane 9). The polypeptide composition of cells (lane 1) and thylakoids (lane 2) are shown for comparison. All lanes contained 10 μg Chl.

Table 3
Summary of electron transport activities

	Rates or calculations[a]	1 M sorbitol, sonication	2 M sorbitol, sonication	2 M sorbitol, protoplast/French press
H_2O to PSI to MV	Rate with MV	0[b]	−10	0
Ascorbate/DCPIP to Cyt c_{553} to PSI to MV	Rate with ascorbate/DCPIP/MV	−19	−10	−17
H_2O to PSI to FeCN	(Rate with 2 mM FeCN)− (Rate with 2 mM FeCN+DBMIB)	(70)−(45) = 25	(57)−(36) = 21	(40)−(25) = 15
H_2O to PSI to 3 mM BQ	(Rate with 3 mM BQ)− (Rate with 3 mM BQ+DBMIB)	ND	(52)−(40) = 12	(52)−(35) = 17
H_2O to PSII to FeCN	Rate with 2 mM FeCN+DBMIB	45	36	25
H_2O to PSII to FeCN (DCMU-insensitive; pre-Q_B)	Rate with 2 mM FeCN+DCMU	37	13	0
H_2O to PSII to FeCN (DCMU-sensitive; Q_B pocket)	(Rate with 2 mM FeCN+DBMIB)− (Rate with 2 mM FeCN+DCMU)	(45)−(37) = 8	(36)−(13) = 23	(25)−(0) = 25
H_2O to PSII to BQ (Q_B pocket)	Rate with 0.5 mM BQ	6	21	24

[a]All rates used in this table were obtained from Table 2.
[b]Rates are given in μmol O_2 mg Chl^{-1} h^{-1}.

(Fig. 5, lane 3) while the doublet at 55 kDa corresponded to ATPase subunits (Fig. 5, lane 4). The presence of the 33-kDa polypeptide of the water oxidation complex (PsbO) was verified using an antibody to the spinach protein (Fig. 5, lane 6) and by direct sequencing [26]. The 23- and 17-kDa proteins of the water oxidation complex were not detected using antibodies raised against the *Chlamydomonas* homologs (not shown), thus supporting the observation that the diatom complex may be more like that of cyanobacteria and red algae than that of green plants. Antibodies to the PsbC (CP43) and PsbB (CP47) polypeptides recognized polypeptides of $\approx$45 kDa and a doublet at $\approx$52 kDa, respectively (Fig. 5, lanes 5 and 6). Lanes 7 and 8 (Fig. 5) were probed using antibodies to the PSII reaction center polypeptides, D1 (PsbA) and D2 (PsbD), revealing polypeptides at 34 and 32 kDa, respectively. Finally, when blots were challenged with an antibody against the 29-kDa LHCP of *Chlamydomonas reinhardtii*, a number of bands from *Cylindrotheca* cross-reacted (Fig. 5, lane 9). These bands have apparent molecular weights (M_r) of 27, 20, 18, 17.5, 17, and 16.5, and have been partially characterized previously [2].

TMBZ-staining for cytochromes (results not shown) revealed bands at 36 and 14 kDa (labeled in Fig. 5) in both heated and non-heated samples. Based on its M_r and stability when heated, we tentatively identify the 36-kDa polypeptide as Cyt *f* (PetA); the identity of the band at 14 kDa is unknown, but it may correspond to Cyt c_{553}, the functional equivalent of plastocyanin in diatoms [9,14]. Overall, these immuno- and histochemical results demonstrated that the abundant proteins visible in our stained profiles (Figs. 4 and 5) correspond to the major photosynthetic proteins expected in a purified thylakoid preparation.

4. Discussion

We previously published a report on diatom LHCPs that included a protocol for purifying thylakoid membranes from diatoms using 0.3 M sucrose [2], but subsequently found these membranes to be inactive in O_2 evolution. Moreover, the membranes lacked PSI activity (ascorbate/DCPIP to MV) and exhibited other characteristics typically associated with disrupted membranes (e.g. altered fluorescence emission properties; Martinson and Plumley, unpublished data). Subsequent experiments indicated that the loss of electron transport activity was related to the use of buffers of low osmotic strength in conjunction with harsh breaking conditions (e.g. passage through a French press at 10 000–18 000 psi, and/or freezing of cells at -60°C prior to breakage). In this communication, we report two protocols for purifying O_2-evolving thylakoids from a diatom. One employed sonication as a means of breaking cells, while the other utilized the ability of some diatoms to form protoplasts under certain growth conditions which, in turn, are readily lysed at low pressure in a French press. The biochemical properties and spectroscopic features of three different thylakoid preparations were compared in an attempt to define the optimum conditions for purifying thylakoids from diatoms. The results showed that use of 2 M sorbitol was necessary for preservation of in vivo spectroscopic properties and electron transport activity. Although rates of activity were low for thylakoids purified using either means of cell breakage, the protocols presented here represent a starting point for the purification of O_2-evolving thylakoids from other diatoms and chromophytes.

4.1. Optimization of thylakoid purification procedure

Purification of O_2-evolving diatom thylakoids required careful attention to two factors: the osmotic strength of the breaking buffer and the conditions used for cell breakage. Studies with crude membranes were done to assess the optimum conditions for the preservation of O_2 evolution. Sorbitol proved to be a very effective osmoticum for use with diatoms as it has for other eukaryotic algae [33] and cyanobacteria (H. Kato and M. Ikeuchi, unpublished results). In addition, O_2-evolving chloroplasts [34,35] have been purified from brown algae using sorbitol. We also found high (>1 M) concentrations of sucrose to be an effective osmoticum with diatom thylakoids, but it was more troublesome to handle. Glycine betaine is another osmoticum that is widely used in photosynthesis research for its stabilizing effect on the O_2-evolving side of the PSII complex [36]. Our results with crude membranes, however, indicated that glycine betaine did not markedly affect the pres-

ervation of PSII activity in *Cylindrotheca* (Table 1). Tests with crude diatom membranes indicated that both MES-NaOH pH 6 or Na-phosphate pH 7 were suitable for use in the sonication procedure, whereas K-phosphate pH 8 gave the best preservation of O_2 evolution in protoplasts (Table 1).

4.2. Characterization of electron transport activity

Thylakoids purified according to the protocols presented in this paper evolve O_2 when supplemented with exogenous electron acceptors such as FeCN, BQ, and MV (Table 2), indicating that the components of the photosynthetic electron transport chain are present and functional. Different electron acceptors function at different points along the electron transport chain, and some may act at multiple locations depending on their concentration (Fig. 1). In light of this, we attempted to calculate the partitioning of electron flow through the electron transport system using inhibitors. Our goal was to try and assess the integrity of PSII and PSI individually.

4.2.1. Estimates of whole-chain electron transport activity and PSI function

Estimates of whole-chain electron transport activity were obtained using either MV, FeCN, or 3 mM BQ (Fig. 1). Since FeCN [30] and BQ [37] can accept electrons from both PSII and PSI, the inhibitor DBMIB was used to determine the partitioning of electron flow through PSII and PSI. With DBMIB added, the rate of O_2 evolution represents electrons flowing to FeCN or BQ through PSII only (Fig. 1). To estimate the electron flow to FeCN or BQ through PSI, the rate with DBMIB was subtracted from the rate without the inhibitor (Table 3). Overall, this method of estimating whole-chain electron transport yielded rates that were similar between the different preparations, and were generally about 10–20 μmol O_2 mg Chl^{-1} h^{-1}. These rates were similar to those observed in thylakoids purified in 2 M sorbitol using sonication with MV as acceptor (Table 2). The other two preparations did not exhibit O_2 uptake with MV alone, but did so when ascorbate/DCPIP were added as donors to Cyt c_{553} (Table 2); the reasons for this remain unclear. From these results, we conclude that all three preparations are capable of whole-chain electron transport, and therefore each has functional PSII, plastoquinone, Cyt b_6/f, Cyt c_{553}, and PSI.

For all three preparations, rates of whole-chain activity were 2.5–100 times lower than those observed in other plants/algae [35,38,39], and four times lower than electron transport rates observed in vivo for *Cylindrotheca* (Table 2). These low rates could be due to damage or loss of one or more of the components of the photosynthetic electron transport chain (Fig. 1). Rates of O_2 evolution with 3 mM BQ or 2 mM FeCN (Table 2) were fairly high in all preparations (40–70 μmol O_2 mg Chl^{-1} h^{-1}) and although these rates are a combination of both whole-chain and PSII-only activity, they do suggest that the water oxidation complex is not the limiting factor in whole-chain electron transport. Moreover, since whole-chain and PSII-only activity both depend on a functional PSII, it seems unlikely that this component is the limiting factor. The integrity of Cyt c_{553} and PSI were assessed using ascorbate/DCPIP and MV (Fig. 1, Table 2). At the low concentration of DCPIP used in this assay (10 μM), electrons are most likely transferred to Cyt c_{553} and then to P700 before transfer to MV [30], thus bypassing PSII, the plastoquinone pool, and Cyt b_6/f. The fact that all three diatom preparations exhibited O_2 uptake with ascorbate/DCPIP/MV (Table 2) indicates that each has functional Cyt c_{553} and PSI. Moreover, the overall similarity between the estimated whole-chain electron transport rates and the rates with ascorbate/DCPIP/MV suggests that loss of Cyt c_{553} or damage to PSI are contributing to the low rates of whole-chain electron transport. If upstream components were the only factor contributing to the low rates, rates with ascorbate/DCPIP/MV would likely be higher.

Cyt c_{553} is readily lost from thylakoid preparations [27,28] and while the low rates of whole-chain electron transport observed here are likely a reflection of this, damage to PSI cannot be ruled out. This is especially true in light of our observation that diatom PSI is remarkably unstable compared to green plant and cyanobacterial PSI ([26,40], Martinson and Plumley, submitted). Work is currently under way to resolve the question of PSI stability in diatoms. Another possible explanation for low rates of PSI-dependent electron transport (Table 3) is that diatom PSI is inaccessible to added electron donors/accept-

ors. For instance, immunocytochemical studies with the diatom *P. tricornutum* have shown that ≈60% of the PSI reaction centers are located in the inner membranes of the thylakoid stacks [6], whereas green plant PSI is localized almost exclusively in the stromal lamellae [7]. If the purified diatom thylakoids retain their stacked configuration, it is possible that endogenous electron donors and/or acceptors are unable to gain access to those PSI reaction centers located in the appressed regions of the thylakoid stacks. At this time, little is known about the forces governing thylakoid stacking in chromophytes and we know of no evidence for the in vivo stacking and unstacking that is characteristic of green plants. Electron microscopic studies on these ultrastructural aspects of purified thylakoids are necessary to address this issue.

4.2.2. Estimates of PSII activity

PSII activity was estimated using FeCN in conjunction with the inhibitors DCMU and DBMIB (Fig. 1). Electron flow to FeCN through PSI is inhibited by DBMIB (Fig. 1) so that the rate of O_2 evolution with both 2 mM FeCN and DBMIB provides an estimate of electron flow through PSII to FeCN. PSII activity ranged from 25–45 μmol O_2 mg Chl^{-1} h^{-1}, with the highest estimates being for thylakoids purified in 1 M sorbitol using sonication (Table 3).

With PSII, FeCN accepts electrons from either Q_B [41] or a site prior to Q_B [42], allowing us to further differentiate total PSII activity into Q_B and pre-Q_B (e.g. DCMU-sensitive and DCMU-insensitive, respectively) components (Fig. 1). The rate of O_2 evolution with FeCN and DCMU (Table 2) represents the DCMU-insensitive component. The amount of electron flow to FeCN that is sensitive to DCMU was calculated by subtracting the rate of O_2 evolution with FeCN and DCMU from the rate of O_2 evolution with FeCN and DBMIB added to the assay (Table 3). The rate with FeCN and DBMIB should be used (rather than the rate with FeCN alone) in order to eliminate any electron flow through PSI to FeCN. From these calculations, we observed that thylakoids purified in 1 M sorbitol using sonication had the highest rates of DCMU-insensitive activity, while thylakoids purified in 2 M sorbitol using the protoplast/French press protocol were completely sensitive to DCMU (Table 3). These results indicated that although PSII activity with FeCN was highest in thylakoids purified in 1 M sorbitol using sonication, there was a substantial amount of disturbance to the reaction center. Estimates of DCMU-sensitive activity with FeCN agreed closely with the values obtained using 0.5 mM BQ, which accepts electrons from the Q_B pocket of PSII [43], and were similar for both thylakoid preparations purified in 2 M sorbitol (Table 3). In addition, the 0.5 mM BQ data agree well with the estimated rate of DCMU-sensitive activity with FeCN for thylakoids purified in 1 M sorbitol using sonication (Table 3), and is consistent with our claim that damage to PSII has occurred in this type of preparation.

4.3. Assessment of protocols

The most significant losses in electron transport activity occurred upon breakage of cells or protoplasts, regardless of the protocol used. A comparison of rates of O_2 evolution measured with BQ at different points during the protoplast/French press procedure (Tables 1 and 2) indicated that the greatest losses occurred upon lysis of the protoplasts (≈47% reduction relative to rates in cells with 0.5 mM BQ) and formation of protoplasts (≈44% reduction). Further purification of crude membranes to yield thylakoids resulted in an additional loss of 18%. In the sonication procedure, substantial losses occurred upon cell breakage (87 and 81% for 1 and 2 M sorbitol, respectively). With 1 M sorbitol, PSII activity was reduced an additional 50% during the purification of crude membranes, whereas activity appeared stable in 2 M sorbitol.

Overall, the best results were obtained using the protoplast/French press method with 2 M sorbitol. Nevertheless, rates of electron transport in purified thylakoids were significantly lower than in vivo rates regardless of the method used to break the cells. To date, we have found sonication and the lysis of protoplasts in a French press to be the only satisfactory methods for expedient lysis of diatom cells. Clearly, no matter how gentle the method of breakage, a significant reduction in electron transport rates occurs. It is possible that addition of some additional component (e.g. cations, salts, chelators) would improve the stability of PSI and PSII function. In ear-

lier studies, we found that addition of 5 mM EDTA and/or 1 mM $MnCl_2$ to the breaking, washing, and/or flotation buffers caused significant alterations in the fluorescence emission spectrum, led to reduced PSII function, and did not increase PSI activity (Martinson and Plumley, unpublished data).

4.4. Spectroscopic characterization

Thylakoids purified in 2 M sorbitol using the protoplast/French press method had fluorescence emission properties most like those observed in vivo, with major emission peaks at 685 and 717 nm (Fig. 3A). Shifts in both the 685 and 717 nm emission peaks were observed in thylakoids purified using sonication, with the greatest deviation from the in vivo spectrum occurring in thylakoids purified in 1 M sorbitol (Fig. 3B,C). In all thylakoid preparations, as well as in cells, the greatest contribution to both emission peaks was made by Chl *c* (i.e. 470 nm, Fig. 3D), indicating the presence of a substantial light-harvesting component in this diatom. Both absorption and excitation spectra revealed an apparent reduction in carotenoids in thylakoids prepared in 2 M sorbitol using the sonication protocol (Figs. 2 and 3D), suggesting that some disruption of the light-harvesting pigment bed may occur with this protocol. In all preparations, there was no evidence of uncoupled Chl *a* (at $\approx$670 nm) or Chl *c* (at $\approx$640 nm) and the accessory pigments, fucoxanthin and Chl *c*, were energetically coupled to the reaction centers. Detailed studies of the fluorescence properties of both cells and purified thylakoids are currently under way.

4.5. Immunological and histochemical identification of photosynthetic polypeptides

The polypeptide composition of the different thylakoid membrane preparations were essentially identical, and all preparations were free of Rubisco (Fig. 4 and not shown). Thylakoids purified in 2 M sorbitol using sonication were characterized in detail using antibodies to an array of photosynthetic proteins (Fig. 5). In general, the results were as anticipated based on our current understanding of oxygenic photosynthesis. A couple of points, however, are worthy of note. The antibody against the PSI complex recognized only 'one' polypeptide on 10–20% acrylamide gels (Fig. 5), but we were able to resolve both the PsaA and PsaB proteins as cross-reacting polypeptides on a 6–12% acrylamide gel ([26]; Martinson and Plumley, submitted). We previously found that the antibody against the 29-kDa LHCP of *C. reinhardtii* recognized polypeptides of 18, 17.5, 17, and 16.5 kDa of *Cylindrotheca* [2] but in this work, two additional polypeptides (27 and 20 kDa) cross-reacted with this antibody (Fig. 5). We attribute the differences to: (1) the use of a different batch of antibody that recognized additional epitopes; and (2) the increased purity of thylakoids in this study. Overall, the immunological data presented in this work confirms the homology of major photosynthetic proteins in oxygenic organisms but, of more importance to this manuscript, supports the claim that our membrane preparation is largely uncontaminated and that the major photosynthetic proteins are present in a non-degraded state.

Two cytochromes were identified in thylakoids purified in 2 M sorbitol using sonication; the active heme in both was stable to heating (not shown). Based on its apparent molecular weight, the 36 kDa polypeptide is most likely Cyt *f*. The identity of the cytochrome at 14 kDa is uncertain, but likely corresponds to Cyt c_{553}. This conclusion is supported by the presence of whole-chain electron transport activity in this thylakoid preparation (Table 2), since Cyt c_{553} is required for this process. In addition to Cyt c_{553}, diatoms contain Cyt c_{550} (17 kDa; [9]), which is chloroplast-encoded in the diatom *Odontella sinensis* [3]. Cyt c_{550} plays a role in O_2 evolution in cyanobacteria [11] and red algae [12], and most likely plays a similar role in diatoms. We were unable to demonstrate the presence of Cyt c_{550} in our thylakoids. However, given the ease with which this protein is lost from cells of the diatom *P. tricornutum* (e.g. freezing in distilled water; [9]), we suspect that both sonication and the process of protoplast formation could lead to loss of this protein in our preparations. Interestingly, we could not detect a 17-kDa cytochrome (i.e. Cyt c_{550}) in cell extracts of *Cylindrotheca* (not shown) suggesting that either this cytochrome is not stained by TMBZ or that the protein was not expressed under the growth conditions employed. Recent work with cyanobacterial mutants has shown that either Cyt c_{550} or the 33-kDa PsbO polypeptide can support O_2 evolution in the absence

of the other [44]. Thus, it is possible that our thylakoids would still retain the capacity for O_2 evolution even in the absence of Cyt c_{550}. Additional studies will have to be conducted to unambiguously determine the presence/absence of c_{550} in our thylakoids and the role of this cytochrome in diatom PSII function. Finally, in addition to the *c*-type cytochromes, one would expect to find Cyt b_6 (18 kDa) and Cyt b_{559} (two subunits of 4 and 9 kDa), which are seemingly ubiquitous among oxygenic photosynthesizers. No bands at these molecular weights could be detected using the TMBZ staining method, probably because *b*-type cytochromes are difficult to detect following SDS-PAGE.

5. Conclusions

Lysis of the silica frustules of diatoms has been a major obstacle in developing methods for purifying O_2-evolving thylakoids from these algae. We have found sonication and French press lysis of protoplasts to be the only expedient means for breaking diatom cells, and, in this paper, present protocols for purification of diatom thylakoids that yield preparations with O_2-evolving activity. Most of the biochemical and biophysical properties of these thylakoids are, as expected, similar to those of green plants/cyanobacteria. Overall, the best results were obtained using the protoplast/French press method with 2 M sorbitol. Although rates of electron transport in purified thylakoids were significantly lower than in vivo rates, these protocols represent a major methodological breakthrough and serve as a starting point for the purification of O_2-evolving thylakoids from other diatoms and chromophytes. Work is currently under way to address questions regarding the stability and functionality of photosynthetic complexes in these ecologically significant algae.

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